Chapter

Evolution of Cartilage Repair Technology

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Abstract

Articular cartilage plays an important role in daily joint activities. With the aging of the social population, the degenerative cartilage injury and the sports injury caused by inappropriate exercise of young patients, etc., the incidence rate of articular cartilage injury is constantly rising, and the injured patients tend to be younger. Although articular cartilage has its corresponding metabolic activities, it is difficult to recover and regenerate itself once it is damaged due to lack of nerve, blood vessel, and lymphatic tissue Common articular cartilage injuries can be divided into three types according to the degree of injury: partial cartilage injury, full-thickness cartilage injury, and osteochondral defect. If partial cartilage damage and full-thickness cartilage damage are not found and treated in time in the early stage, further deterioration will lead to serious osteochondral defects. After the corresponding subchondral bone injury, the upward invasion of the upper cartilage layer will also cause the overall osteochondral injury. Therefore, whether the osteochondral injury caused by the top-down or the osteochondral injury caused by the bottom-up, it seriously affects the normal activities of human joints. It not only brings great inconvenience to the daily life of patients, but also causes huge economic and psychological burden to patients. At the same time, it also consumes a large number of social public medical resources. Therefore, seeking an effective osteochondral repair strategy is not only the urgent need and hope of the society, but also one of the clinical scientific problems that clinicians and scientists urgently need to solve.

Keywords: articular cartilage, osteochondral defect, scaffold, autologous chondrocyte transplantation technology

1. Introduction

The microfracture technique creates a channel between the cartilage defect and the underlying bone marrow by opening the subchondral bone [1]. At present, it is generally believed in clinical research that pluripotent bone marrow mesenchymal stem cells are released and recruited to the defect site through these channels to repair articular cartilage. This technology is often used in clinic because of its simplicity, rapidity, and low cost. However, this method is only effective for small defects, and cannot form hyaline articular cartilage after repair. It can only provide relative

functional improvement after operation, and its clinical effect and significance are relatively limited.

2. Osteochondral transplantation

Autogenous osteochondral transplantation is also used in clinical practice. It is to take out cylindrical osteochondral tissue from the cartilage surface of the non-load bearing area of the joint and implant it into the cartilage defect of the load-bearing area. Although it has been reported in the literature that good clinical results can be obtained by autologous bone and cartilage transplantation, the results vary greatly depending on age, sex, and lesion size. At the same time, the injury and discomfort of the donor site and the limited availability of local donor tissue make the autologous bone and cartilage transplantation only suitable for some small- and medium-sized cartilage defects. At the same time, there are problems in the repair and healing of cartilage between the transplanted bone and cartilage, and the healing of the transplanted cartilage and the surrounding cartilage of the recipient area. In addition, the adverse effects of the wound opening caused by the injury of the donor area and the release of bleeding inflammatory factors on the microenvironment and homeostasis of the joint make its application limited and gradually reduced. Although allogeneic osteochondral transplantation does not have the problems of donor damage and insufficient graft size, there are problems such as the preservation of allografts, the availability of tissues, the immune response of recipients, and the shortage of donor sources and quality that are also practical problems in clinical application.

3. Scaffold

In recent years, with the emergence and continuous development of tissue engineering regenerative medicine, it has brought new hope for the repair and regeneration of bone and cartilage after injury. Tissue engineering regenerative medicine mainly includes three factors: biological scaffolds, seed cells, and growth factors [2]. The scaffold of cartilage tissue engineering technology is equivalent to the extracellular matrix. It should be non-toxic, not causing inflammation, and has high porosity. It can provide a good microenvironment for cell growth and can still maintain its shape after implantation [3]. The earliest scaffold materials used in cartilage tissue engineering are PGA, PLA, PLGA, etc. [4]. When PRP gel is used as a scaffold alone, it is easy to squeeze and deform after subcutaneous implantation, and specific cartilage tissue cannot be formed [5, 6]. 3D printing technology can be used to prepare high-precision scaffolds [7, 8], Plga/dacecm tissue-engineered cartilage scaffolds were prepared by low-temperature deposition 3D printing technology [9, 10], and the results showed that the scaffolds were not cytotoxic and has excellent performance [11]. Lin's [12] study also confirmed that this scaffold can better promote the proliferation of bone marrow mesenchymal stem cells and promote the formation of new cartilage. By integrating the preparation process, it will be a new research direction to construct tissue engineering cartilage scaffolds that cannot only recruit endogenous stem cells but also facilitate the maturation of new tissues.

The first generation of autologous chondrocyte transplantation technology is to take articular cartilage under arthroscopy, isolate chondrocytes, culture and

expand them *in vitro*, inject cell suspension into the defect, and finally cover with autologous periosteum for suture. This method can repair cartilage damage with a depth of more than 6–8 mm [13]. However, there are also many disadvantages, such as the leakage of cell suspension and the proliferation of periosteum, which require arthroscopic surgical resection [14].

The second generation of autologous chondrocyte transplantation technology is to use some biofilms, such as collagen membrane, to suture with surrounding tissues instead of periosteum. Although the cost is higher than that of autologous periosteum, it does not require secondary surgery, so it is more economical [15].

The third-generation matrix-induced autologous chondrocyte transplantation is to expand the chondrocytes cultured *in vitro*, implant them into the rough surface of the I/III double-layer collagen membrane, and then replant them. Finally, the more biocompatible fibrin glue is used for adhesion, eliminating the need for suturing, making the operation easier and reducing the risk of cell leakage [16].

The fourth-generation cartilage repair technology With the continuous development of cartilage repair technology, after the iterative upgrading of the previous three generations of cartilage repair technology, cartilage repair technology has reached a new height. Although the third-generation cartilage repair technology overcomes the shortcomings of the previous two generations of repair technology, such as poor survival of cells in the defect area, poor long-term repair effect, uneven articular surface, and so on, some problems in the past have not been solved. For example, articular cartilage is composed of hyaline cartilage, which is still replaced by fibrocartilage after repair, resulting in unsatisfactory mechanical properties and biological properties.

How can we repair cartilage damage and solve these problems at the same time? With the further in-depth study of the cell microenvironment, it is found that the principle of microfracture technology, for example, is to introduce stem cells in bone marrow into the cartilage defect area, so that the stem cells can grow, and differentiate and repair in the microenvironment of the cartilage itself, playing a good repair effect. It is the so-called "one side of the soil nourishes one side of the people." The scaffold we made not only carries the cell load, but also wants it to grow and differentiate well on the scaffold, which has been imitating the internal environment of the machine. So why not use the microenvironment of the cell itself to make this cell carrier? Therefore, the concept of extracellular matrix (ECM) came into being. It can not only provide a good microenvironment for cell growth and differentiation, but can also solve the problems of cell metabolism and biomechanical properties.

3.1 Extracellular matrix (ECM)

Extracellular matrix (ECM) is a matrix structure that is synthesized and secreted by cells and distributed on the cell surface or between cells. It is a complex network composed of proteins and proteoglycans, which can provide support for tissues and regulate cell functions. It is the dynamic microenvironment of the stem cell niche. Therefore, it has attracted the continuous attention of tissue engineering researchers [17, 18]. In addition, cartilage extracellular matrix can activate intracellular signal transduction pathways through various growth factors and cytokines [19]. Due to the complex composition of extracellular matrix, it is almost impossible to fully bionize the cartilage extracellular matrix in terms of composition, morphology, and function in the current progress of cartilage tissue engineering. At present, the bionic biological scaffold materials used in cartilage tissue engineering include extracellular

matrix-derived materials and non-extracellular matrix-derived materials. The extracellular matrix-derived materials include simple-component materials extracted from the extracellular matrix, mixed-component materials, and tissue acellular extracellular matrix materials. The extracellular matrix-derived material is closer to the cartilage extracellular matrix than other materials in composition and is a very excellent biomimetic scaffold material.

1. The composition of extracellular matrix determines the function of extracellular matrix, such as providing support for cells, regulating the dynamic behavior of cells and intercellular communication [20, 21]. The composition of extracellular matrix is different in different tissues [22]. The main components of cartilage extracellular matrix are collagen, proteoglycan and other non-collagen and glycoproteins, as well as a various growth factors, cytokines, and proteases. Collagen is a very important and most abundant macromolecular component of the extracellular matrix of cartilage. In articular cartilage, type II collagen accounts for 90%–95% of the total collagen. The main purpose of collagen is to provide tension and shear force for tissues and to fix proteoglycans in the matrix [23]. Proteoglycan is a macromolecule in the extracellular matrix of cartilage, which is second only to collagen. It is a covalent conjugate composed of glycosaminoglycans and core proteins [24]. Glycosaminoglycans are composed of long-chain unbranched-repeating disaccharide units. Chondroitin sulfate, keratin sulfate, and dermatan sulfate are glycosaminoglycans that covalently bind with core proteins to form proteoglycans in cartilage, of which chondroitin sulfate accounts for 55–90%. 80–90% of proteoglycans in articular cartilage form large polymers, which are called polyproteoglycans. Polyproteoglycan and hyaluronic acid (the only glycosamine polysaccharide that does not undergo sulfation) can bind with connexin in a non-covalent bond to form a stable polyproteoglycan hyaluronic acid connexin complex. The non-covalent binding force between these complexes is very strong, and only proteolytic enzymes can degrade it. In the matrix, polymerization stabilizes polyproteoglycans. Other ingredients include elastin, which forms a network of elastic fibers and gives the tissue elasticity; fibronectin can connect cells to the extracellular matrix; cartilage oligomeric protein, which only appears in cartilage, has the ability to connect chondrocytes and a small amount of lipids. Cartilage extracellular matrix also stores many growth factors and cytokines, bone morphogenetic protein, insulin-like growth factor, basic fibroblast growth factor, platelet-derived growth factor, and chondromodulin, and forms a good storage pool to store them. Changing the conditions can activate the activities of special enzymes, cause the release of factors in the storage pool, and achieve rapid and stable regulation of cell functions [25–27].

2. Extracellular matrix-derived scaffolds in cartilage tissue engineering should simulate the extracellular matrix of cartilage in structure and composition, and provide an ideal microenvironment for the proliferation and differentiation of seed cells. Natural polymer scaffold materials are derived from the organism itself and have the advantages of good biocompatibility, low cytotoxicity, and easy degradation, and the degradation products are easily absorbed by the human body without inflammatory reaction [28, 29]. Extracellular matrix-derived scaffolds are scaffolds made of one or more components of extracellular matrix, which are derived from extracellular matrix and are closer to cartilage tissue than other biomaterials. The commonly used extracellular matrix source scaffold

materials include collagen, glycosaminoglycan, hyaluronic acid, gelatin, chondroitin sulfate, tissue acellular extracellular matrix, etc. These materials can be used alone to make scaffolds, but it is often difficult to imitate all components of the cartilage when applied alone. Therefore, some researchers have also combined two or more of these materials, or combined with other natural materials or artificial materials [30, 31]. Extracellular matrix-derived scaffolds can be divided into four categories according to their composition: monomeric natural polymer materials, multiple natural polymer mixed materials, new biomaterials constructed by combining natural polymer materials with synthetic polymer materials, and tissue acellular extracellular matrix materials.

3. Preparation of tissue acellular extracellular matrix scaffold: Cartilage tissue needs to go through two very important steps, tissue acellularization and scaffold preparation, before it can be prepared as a scaffold derived from extracellular matrix. The purpose of tissue decellularization is to remove the substances that cause immune reaction, such as cell membrane materials, soluble proteins, nucleic acids,, while retaining the extracellular matrix components of cartilage as much as possible and maintaining its biological activity. At present, there are two kinds of commonly used decellularization methods: physical method and chemical method. The physical methods include freezing and thawing method, mechanical oscillation method, differential centrifugal method, etc. Chemical methods include enzymatic digestion, high or low osmotic solution decellularization, acid-base decellularization, etc. Usually, one method or a combination of methods is used in the process of decellularization. When preparing acellular extracellular matrix materials, more attention should be paid to whether acellularization is complete and the loss of components. When the extracellular matrix material is prepared, it needs to be made into a scaffold. At present, many methods have been developed to prepare cartilage tissue engineering scaffolds. Different preparation processes can have obvious effects on the performance of scaffolds. In practical application, different preparation processes can be selected according to the conditions [29]. Freeze drying method: The extracellular matrix slurry is poured into the mold, freeze-dried at low temperature, then cross-linked, and finally sterilized to form a 3D scaffold. Immunogenicity and toxicity are removed. Cartilage acellular extracellular matrix scaffold material has many advantages. It is completely derived from biological tissue. After acellular treatment, the immunogenicity is removed to the maximum extent, and many effective components of natural cartilage extracellular matrix are retained. It can provide a better microenvironment for seed cell growth than pure component materials and artificial mixed materials. The extracellular matrix contains natural cytokines, which can promote the proliferation and differentiation of seed cells without adding exogenous cytokines. The PLA General Hospital adopted its own unique preparation process to form a biphasic-oriented stent. The cells implanted in the scaffolds were induced to differentiate into chondrocytes and grow in the direction induced by the scaffolds. Finally, the regeneration and growth of the repaired cartilage become similar to the natural cartilage.

The prepared scaffold had three-dimensional porous sponge-like longitudinally oriented structure. There were cartilage fibers around the scaffold pores. After hematoxylin-eosin staining, no nucleus was observed. Both safranin O and Sirius red staining confirmed that cartilage tissue engineering scaffold contained collagen and

cartilage matrix. The porosity and water absorption of the scaffold was (91.8 ± 2.9)% and (93.5 ± 1.4)%, respectively. MTT results showed that the leaching liquor of human cartilage-derived extracellular matrix was non-toxic to chondrocytes. After co-culture, human chondrocytes adhered, proliferated, and evenly distributed on the peripheral wall of the scaffold pores. The results showed that human articular cartilage-derived extracellular matrix had similar composition to natural cartilage, provided the structure suitable for cell adhesion and proliferation, and exhibited good histocompatibility. Therefore, human articular cartilage-derived extracellular matrix can be used as a scaffold material for repairing cartilage defects by tissue engineering technique.

1. Clinical application and effect of ECM stent. The fourth-generation cartilage scaffold developed by the Orthopaedic Research Institute of the General Hospital of the Chinese people's Liberation Army combined with autologous chondrocytes to repair the local cartilage defect of the femoral condyle has achieved very good results after more than 10 years of clinical verification. It has been popularized in clinic.

2. For summary in recent years, with the rapid development of cartilage tissue engineering, the preparation and selection of scaffold materials have become a hot topic for domestic and foreign scholars. From the current situation, great progress has been made in the research of cartilage tissue engineering scaffold materials. At present, it is recognized that the ideal biomimetic scaffold for cartilage tissue engineering should have the following characteristics: It simulates natural car-

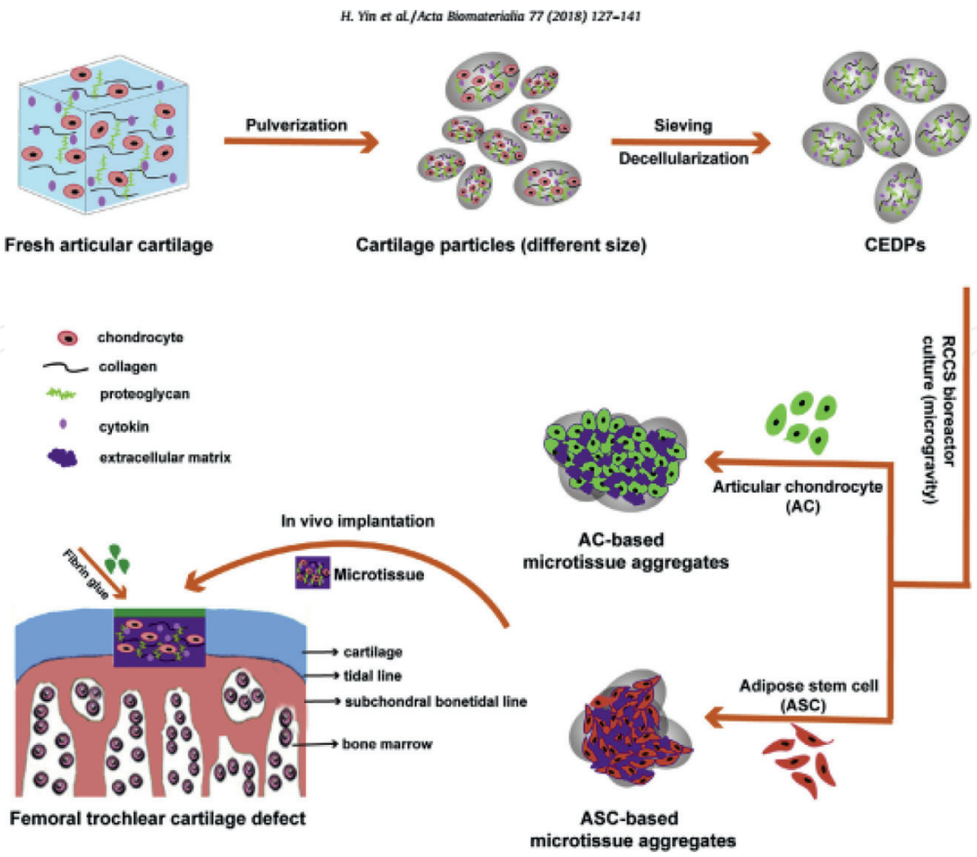

Figure 1.
ECM scaffold preparation, composite cells, cartilage defect model, and treatment plan [33].

tilage tissue components, has good proliferation and differentiation promoting effects on seed cells, has biomechanical characteristics close to cartilage, and can be degraded *in vivo* and will not cause adverse reactions. Extracellular matrix-derived scaffolds have been used in cartilage tissue engineering and clinical practice because of some characteristics of biomimetic scaffolds, and have been proved to be good biomimetic scaffolds for cartilage tissue engineering. However, the shortcoming is that the scaffold materials derived from extracellular matrix have poor mechanical properties and are not easy to be processed. Extracellular matrix as a scaffold material also has its disadvantages. Because there is almost no way to maintain the original physical properties of cartilage tissue during the fabrication process, the fabricated extracellular matrix scaffold cannot reach the level of natural cartilage in terms of biomechanical properties, and atrophy occurs after seed cells are planted. However, these problems can be greatly improved by using cross-linking method during the fabrication of scaffold. Rowland et al. [32] analyzed the effects of heat crosslinking treatment, ultraviolet irradiation, and chemical crosslinking agent carbodiimide on the contraction of scaffolds, combined with adult bone marrow-derived stem cells for chondrocyte differentiation culture, and proved that crosslinking (non-crosslinking as control) can prevent cell-mediated contraction of extracellular matrix scaffolds (**Figures 1-4**).

Therefore, the future research direction must focus on making full use of the existing materials, continuously improving the preparation process, combining synthetic materials with extracellular matrix-derived scaffold materials to prepare

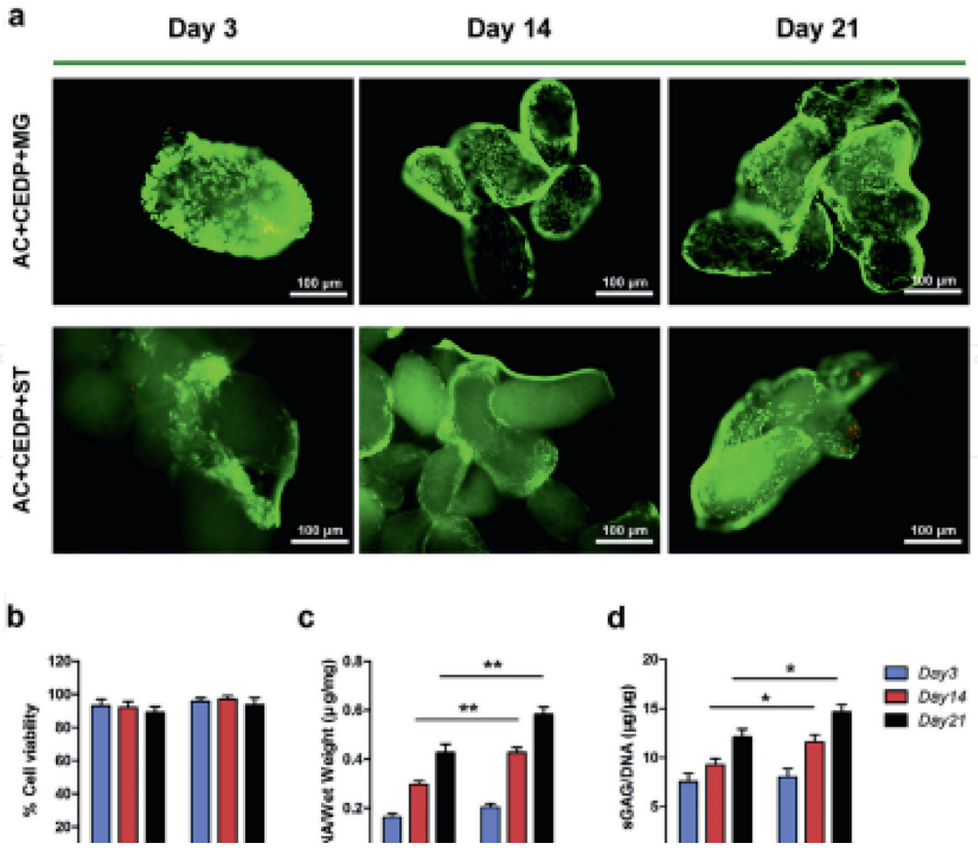

Figure 2.
ECM scaffolds after decellularization of cartilage tissue, and then recombined with autologous chondrocytes or adipose stem cells [33].

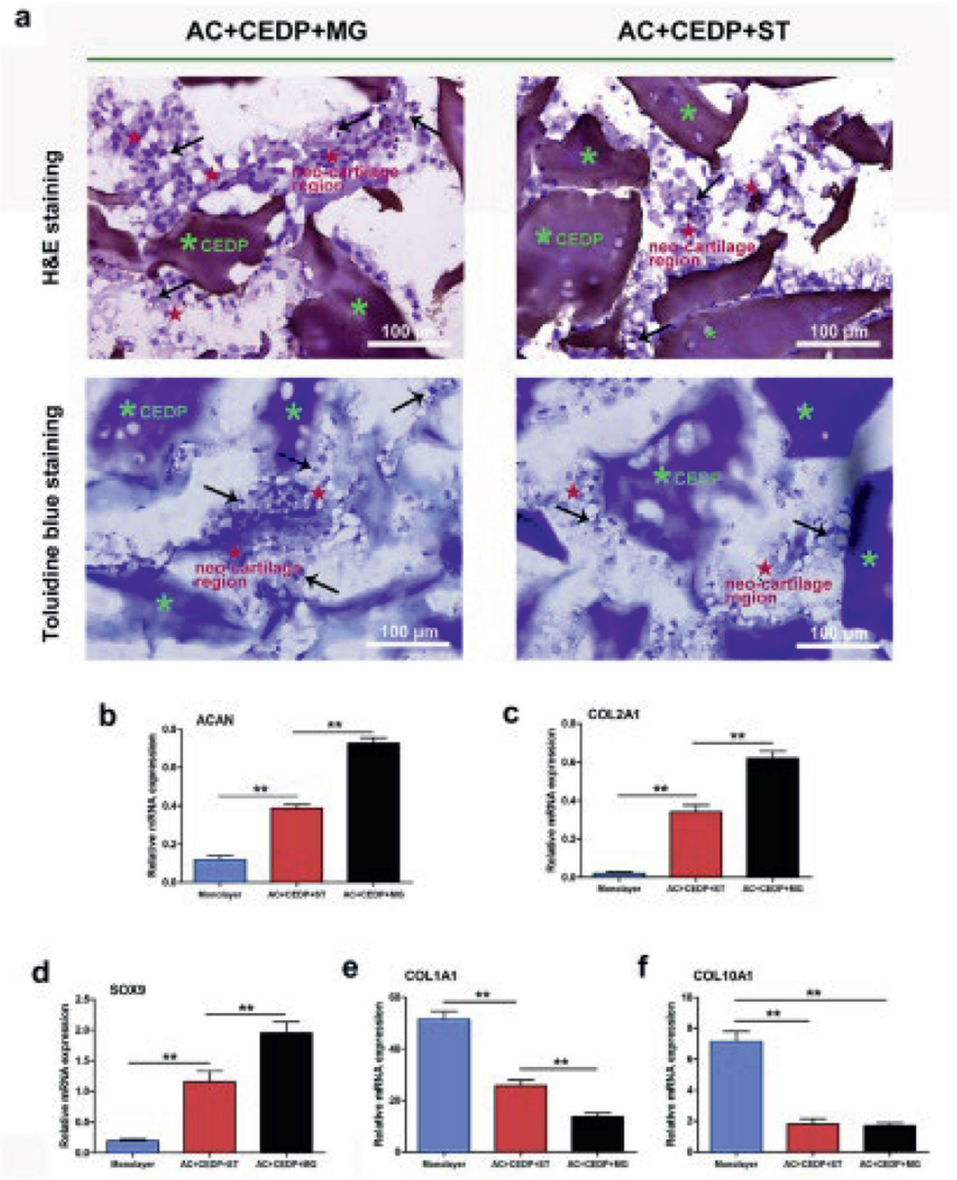

Figure 3.
The ECM scaffolds of cartilage tissue after decellularization treatment were then compounded with autologous chondrocytes or adipose stem cells ECM scaffolds. After the cells were compounded, cell viability and toxicity tests showed that the cells survived well and had strong activity on the scaffolds [33].

cartilage tissue engineering scaffolds, and further exploring methods to change the properties of various materials. That is, the fifth-generation scaffold micro-tissue composite biomimetic scaffold mentioned later (**Figures** 5-7).

Regeneration of articular cartilage is one of the most serious problems facing joint surgeons. In recent years, microcarrier applications have made great progress in cartilage tissue engineering. One advance is the cost-effective expansion of seed cells that provide the necessary microenvironment for cells. Furthermore, microcarriers can also carry proteins, factors that are beneficial for cartilage repair and drugs for cartilage regeneration. Some microcarriers have the advantages on injection. The use of microcarriers having these features avoids the disadvantages of conventional methods and provides unique advantages. For clinical transformation potential, microcarriers have many advantages, such as supplying plenty useful cells, factors, drugs,

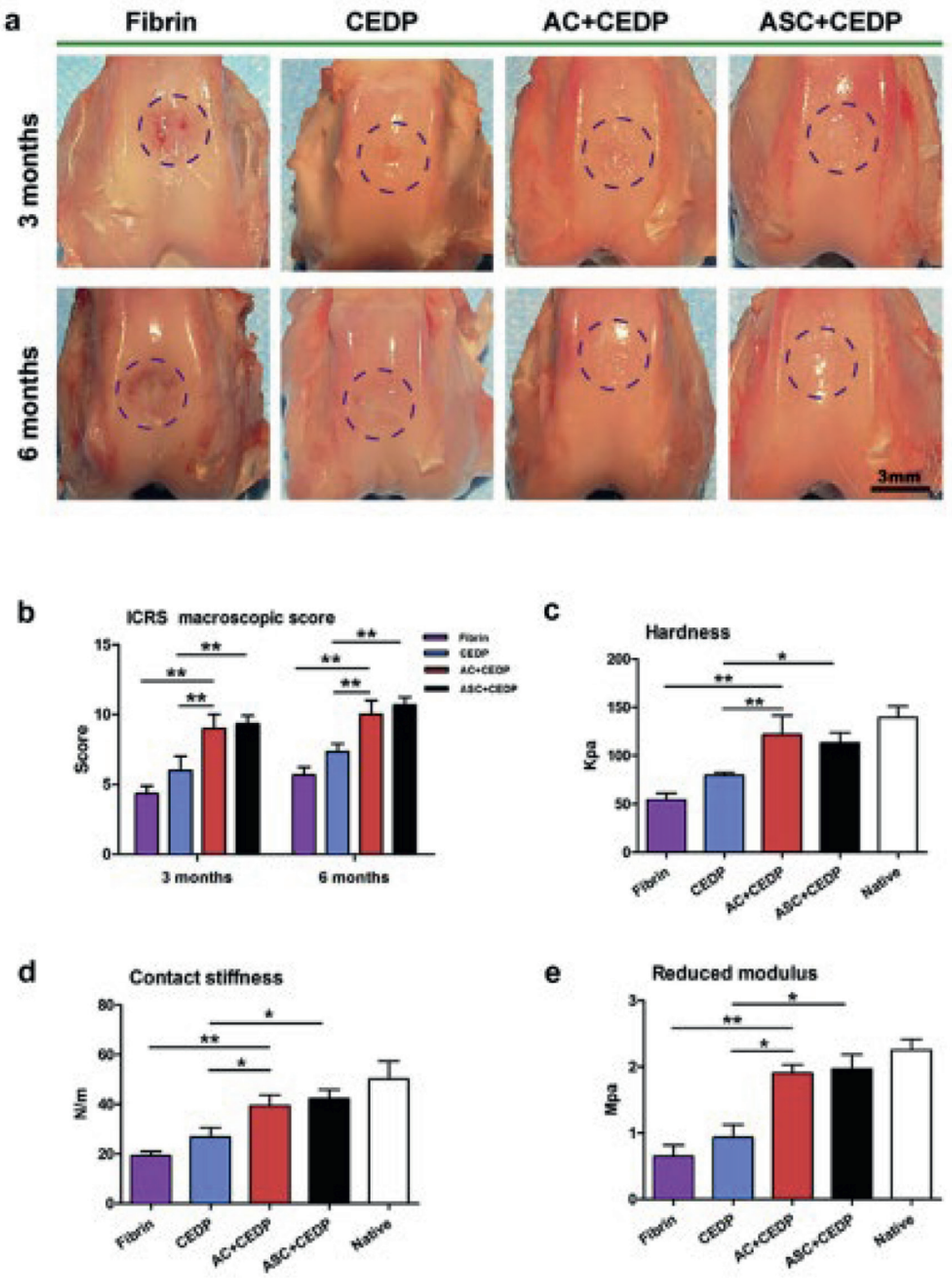

Figure 4.
The ECM scaffolds after decellularization of cartilage tissue, and then composite with autologous chondrocytes or adipose stem cell ECM scaffolds and composite cells to repair the cartilage defect of femoral condyle in rats [33].

microenvironment. Microcarriers also have many application features. First, they can be injected directly into the corresponding site to weaken invasiveness. Second, they can be implanted after organoid formation to enhance repair efficiency. Finally, combining with scaffolds can meliorate the mechanical deficiencies of microcarriers. Thence, the application of microcarriers has great potentiality for clinical translation. A brand new application of microcarriers on tissue engineering is to place them inside hydrogels to make scaffolds or bioinks. Tissue engineering may revolutionize the status quo of cartilage regeneration. However, achieving clinical translation still requires a lot of research support.

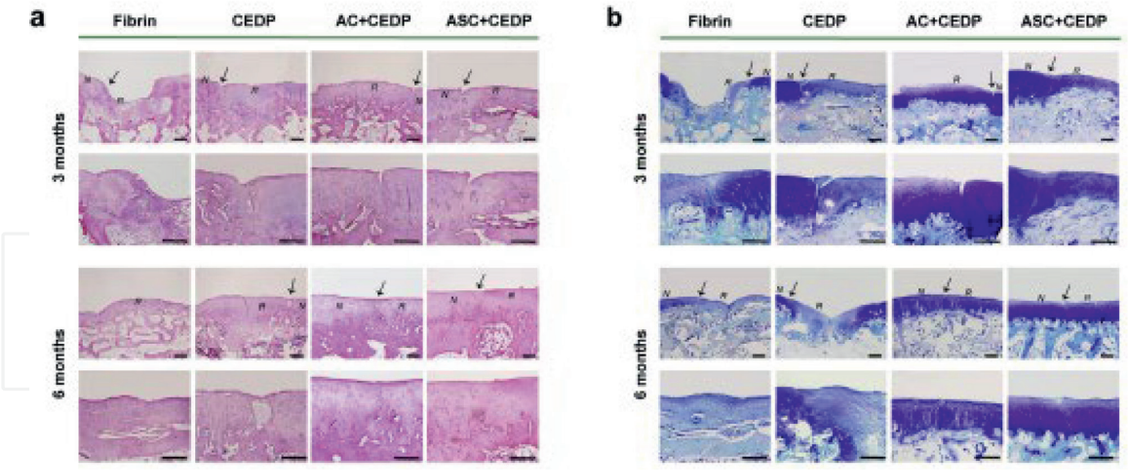

Figure 5.
Pathological section of cartilage defect repaired by ECM scaffolds after decellularization of cartilage tissue and then compounded with autologous chondrocytes or adipose stem cell ECM scaffolds [33].

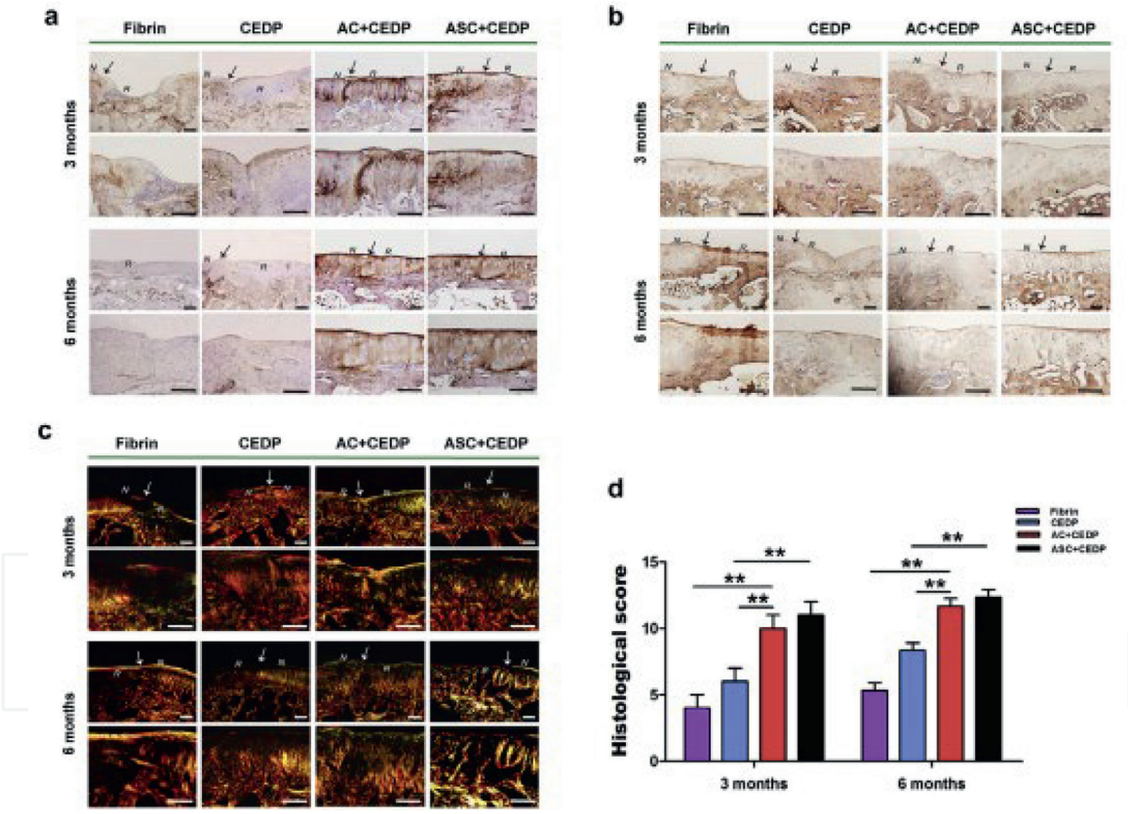

Figure 6.
Pathological section of cartilage defect repaired by ECM scaffolds after decellularization of cartilage tissue and then compounded with autologous chondrocytes or adipose stem cell ECM scaffolds [33].

The advantages of microcarriers are discussed by comparing the characteristics of the microcarrier with other traditional methods. We also discuss the utilization potentiality of the microcarrier and the prospect of future development.

Articular cartilage is very important in the human body. Due to the avascular nature of articular cartilage, it is basically unable to achieve self-healing. Therefore, the treatment of articular cartilage damage is a serious problem for orthopedic

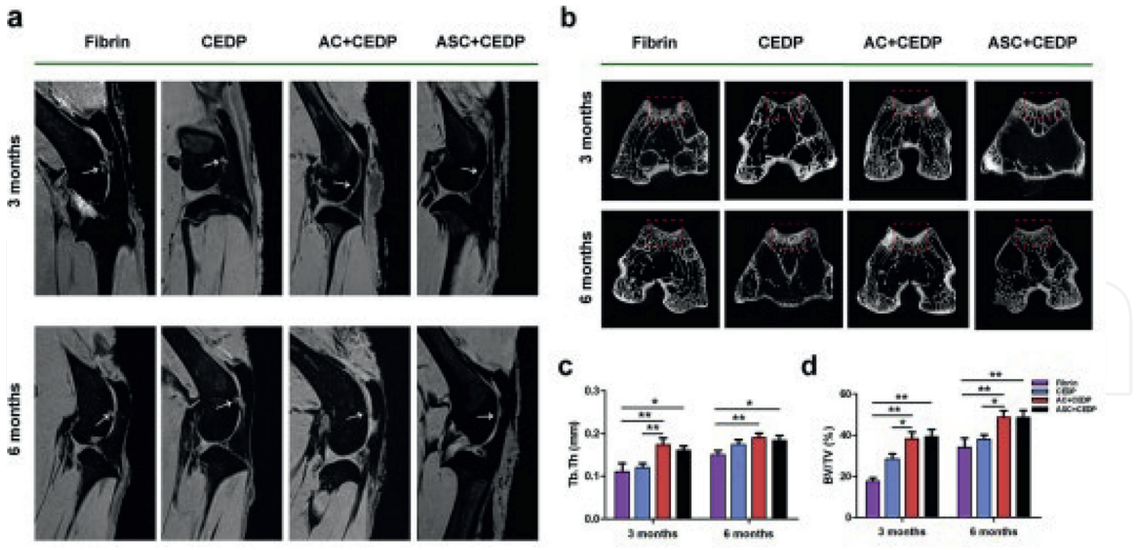

Figure 7.
MRI image of cartilage defect repaired by ECM scaffolds after decellularization of cartilage tissue and then compounded with autologous chondrocytes or adipose stem cell ECM scaffolds [33].

surgeons. If not treated properly, articular cartilage defects can easily lead to osteoarthritis or accelerate the progression of osteoarthritis (OA). OA is one of the most common degenerative joint diseases, and its most notable features are pain and limited joint mobility. The most commonly used methods for clinical treatment of cartilage injury today include microfracture and surgical lavage. Traditional cartilage repair techniques in clinical treatment include bone marrow stimulation techniques (BMS), synthetic, etc. However, all these traditional techniques have shortcomings, such as the inability to repair large-scale cartilage damages, the high rate of secondary operations, and the unsatisfactory prognosis. Tissue engineering (TE) technology has developed speedily in recent decades. Accordingly, a new method for cartilage defect repair is provided. However, traditional tissue engineering techniques have three drawbacks in cartilage regeneration. First, chondrocyte loses certain phenotype in *in vitro* expansion. Second, traditional three-dimensional porous scaffold has hollow phenomenon. Third, tissue-engineered cartilage takes a long period of time to repair the cartilage defect in *in vivo* models. In the field of tissue engineering for cartilage regeneration, many progress have been made in the study of cartilage repair using microcarriers. A microcarrier is a microparticle that can carry cells, factors, or drugs, with a diameter of about 100–300 microns [34]. Microcarriers provide some new ideas for clinical treatment of cartilage injury-induced OA.

4. Traditional methods for treating cartilage injury and characteristics of microcarrier methods

The function and quality of articular cartilage deteriorates with age. Cartilage damage usually progresses from the surface of the articular cartilage to the subchondral bone, leading to the generation of OA.OA creates a huge economic and social load. Degenerative joint damage is often accompanied by cartilage loss. The study reported that cartilage defects in OA patients with symptoms for more than 2 years were more likely to have accelerated progression.

Hence, the treatment of articular cartilage damages is especially important. The traditional techniques commonly used are as follows: first, BMS technology; second, filling cartilage damages with biological tissue; third, cartilage cell implantation; fourth, use metal or other artificial materials to repair cartilage defects; fifth, cell therapy; sixth, drugs that stimulate cartilage repair.

Microfracture technique is a common technique for repairing cartilage damage in BMS technology. It repairs bone marrow defects by transferring bone marrow mesenchymal stem cells (BMSCs) from the intramedullary cavity to the surface of the cartilage damage through fracture drilling. However, the chondrogenic potential of BMSCs is significantly reduced in the elderly [35]. If the cell source is coupled with the disease state, the repair effect is greatly diminished. Therefore, "vivacious" BMSCs are at the heart of the microfracture technique approach for cartilage repair. The use of autologous cartilage as a biological tissue to fill cartilage defects is also a representative method to repair cartilage damage [35]. Osteochondral transplantation can effectively treat knee joint cartilage friction injury. Symptoms of successful transplant patients improved significantly, but this method does not guarantee a good prognosis and a high success rate. However, not only does the surgery have a low success rate, but autologous cartilage transplantation itself is a method of repairing damaged cartilage by destroying healthy cartilage. Fresh allogeneic osteochondral grafts are primarily used to treat young patients with extremely severe articular cartilage damage. Although allogeneic cartilage transplantation has been reported to relieve symptoms of cartilage damage, the scarcity of donor sources has made it impossible to expand the scope of this technique.

Finding a viable treatment to repair damaged cartilage before osteoarthritis develops has become a worldwide problem. Cartilage TE technology emerges as a new proven treatment that offers hope to those who need to treat articular cartilage injuries. Cartilage TE uses cell differentiation and proliferation factors to culture and expand cartilage seed cells *in vitro*, co-culture high-quality seed cells with bioscaffold materials, fill them into the damaged area of cartilage, and gradually combine with the original cartilage to form new cartilage tissue.

Microcarriers [34] are a type of small functional particles with a diameter of about 100–300 μm. Microcarriers contain a wide variety of materials with good biocompatibility, which can boost seed cells as a suitable support matrix. Compared with the traditional methods, functional TE microcarriers can repair cartilage damage, which can not only play the unique advantages of microcarriers, but also avoid some disadvantages of traditional methods.

Advantages of microcarriers over conventional techniques: First, an important advantage of microcarriers is that they can be loaded with sufficient seed cells, which expand *in vitro*. More BMSCs are carried on microcarriers than BMSCs released from BMS. If microcarriers are fabricated by electrospray method, a large amount of BMSCs can theoretically be carried [36]. Second, the microcarriers made of high-quality biomaterials also take full advantage of the traditional method of filling cartilage defects with biomaterials. Second, microcarriers can also be used to treat cartilage damage by using traditional methods of filling cartilage defects with biomaterials. For example, alginate-based microcarriers resemble extracellular matrix (ECM) and can promote cartilage regeneration even without seed cells. Third, in a sense, microcarriers also belong to an artificial material. High-quality microcarriers with seed cells are cultured to constitute tissue-engineered cartilage microtissues. Microtissue [37] provides cartilage tissue to damaged areas of cartilage in a manner similar to cartilage grafting. The application of microcarriers has

opened up a new way for the treatment of cartilage injury by the method of artificial material implantation. Fourth, microcarriers can target delivery of drugs such as chondroitin sulfate and glucosamine sulfate that are beneficial for the treatment of cartilage damage.

5. Summary of the advantages of microcarriers in the treatment of articular cartilage injury

TE aims to develop biological products to replace damaged tissues or organs with the goal of restoring normal function. Current TE therapies for cartilage regeneration are dominated by the use of synthetic or natural implants. A sufficient amount of high-quality seed cells and a proper supporting matrix are the basic conditions for TE. In the field of TE to repair cartilage damage, microcarriers have advantages in every condition. The advantages of microcarriers from these aspects are summarized below.

5.1 Adequate and "virant" seed cells

Adequate and high-quality seed cells are one of the important components of TE. Microcarriers can carry seed cells and target them to the damaged site for repair. BMSCs have been intensively studied, and many are devoted to their application in cartilage damage repair. BMSCs have been shown to possess immunomodulatory functions, multilineage differentiation potential, and tissue homing properties. Furthermore, it is worth noting that even BMSCs obtained from severe OA patients have chondrogenic capacity and can synthesize cartilage extracellular matrix. Studies [36, 37] pointed out that microcarriers can carry a large number of BMSCs and make them successfully differentiate into chondrocytes, and finally form cartilage TE implants. These studies demonstrate that the microcarriers loaded with BMSCs can be used for cartilage damage repair both *in vitro* and *in vivo*.

Human adipose-derived stem cells (hADSCs) are also able to differentiate into cartilage. A study [38] reported that amplifying ADSCs on microcarriers and then implanting them into defects could well promote cartilage repair. One article [39] demonstrated that rapidly degrading microcarriers promoted cartilage regeneration by promoting the generation of immature bone-like tissue. Another study [40] described the effect of different cell densities and different differentiation states of MSCs in microcarriers on cartilage repair. This research shows that the high density of mesenchymal stem cells is beneficial for cartilage repair, and the well-differentiated state of MSCs also has an important impact on cartilage repair. In another study, the combination of hADSCs and transforming growth factor-beta (TGF-β) 3 microcarriers was much more effective than alone in a rabbit model of OA treatment.

Chondrocytes clearly promote cartilage regeneration [38]. There was no significant difference in yield between chondrocytes carried in dynamic microcarriers and chondrocytes produced in tissue culture plates.

In addition, the seed cells carried by the microcarriers can stay in the cartilage defect for a longer time than the mesenchymal stem cells in the BMS method. One study [37] showed that microcarrier-carried cells or their progeny cells persisted for at least 6 weeks.

5.2 Microenvironment

The microenvironment is also one of the conditions affecting TE. Microcarriers can provide a favorable microenvironment for cartilage defect repair, such as providing an appropriate matrix to help seed cell adhesion and proliferation or carrying cytokines that promote cartilage formation. The microenvironment provided by the microcarriers also has an impact at the protein level, which can guide seed cells into the cellular matrix, which can then be used to restore damaged cartilage.

The results of genetic analysis showed that the chondrocyte phenotype of chondrocytes expanded by dynamic microcarrier culture did not disappear. In a study of chondrocyte expansion methods using dynamic microcarriers, it was found that it could increase the expression levels of hyaline cartilage proteins such as Col2 and Agg compared with the traditional 2D cultures.

Biomaterials such as gelatin can provide a microenvironment conducive to seed cell proliferation and adhesion. According to research [36], microcarriers containing gelatin can be used to repair cartilage damage because they can promote the proliferation of seed cells.

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ECM proteins also play an important role in promoting cartilage regeneration. Microenvironments maintained by high-quality biomaterials, such as alginate hydrogels, also have the potential to repair cartilage damage due to their properties similar to natural ECM. Biomaterial-based approaches, such as the use of pre-formed hydrogel matrices, can provide mechanical stability while also taking advantage of biochemical incentives to maintain cellular propensity for chondrogenic differentiation. Alginate is very suitable as a raw material for the manufacture of microcarriers. A previous study [41] noted that alginate hydrogel microcarriers provided ECM in chondrocyte culture, promoting cell differentiation toward cartilage, compared to conventional 2D cultures with or without TGF-β1 added. The internal structure of these microcarriers is very similar to that of natural ECM, so they can be used as an alternative ECM for implantation in cartilage defect areas (**Figures 8-10**).

The application of appropriate matrix assistance can provide a better microenvironment for cells to differentiate into cartilage. In one study, Ramkumar used microcarriers composed of agarose (AG) and collagen type II (COL-II) to carry bone marrow mesenchymal stem cells. COL-II not only promotes mesenchymal stem cell proliferation but also increases local ECM content [32]; therefore, microcarriers prepared with COL-II and agarose can mimic some of the protein components in ECM to promote cartilage repair. The microspheres prepared by the authors are 80–100 microns in diameter, which does not exactly match the size of the defined microcarriers, but produces basically the same effect. Lineage-specific differentiation of embedded BMSCs did not disappear in culture. More importantly, the microcarriers prepared with COL-II promoted the expression of the chondrogenic phenotype of BMSCs. Furthermore, the microcarriers have excellent physical properties and do not have any negative effects on cell viability. In a study [44], Paulomi Ghosh's group and his team used microcarriers to carry acellular cartilage for related research. The advantage of adding acellular cartilage to microcarriers is that acellular cartilage itself contains a large number of chondrocytes' own proteins, cytokines, GAGs, and TGF,

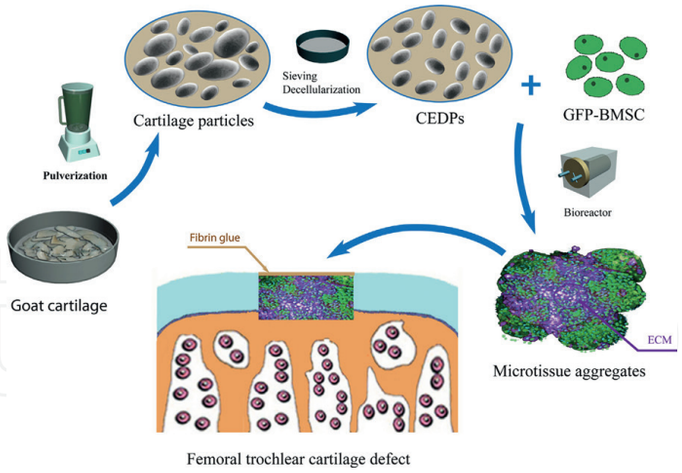


Figure 8.
Flowchart of the experimental design of the fresh goat knee cartilage made into pellets through a series of procedures and co-cultured with BMSCs for repairing rat cartilage damage. [42].

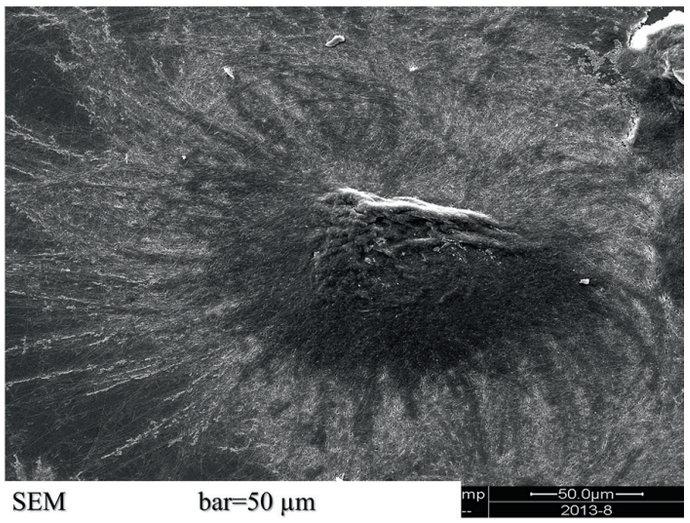


Figure 9.
Scanning electron microscope observation of cartilage-derived microcarriers Villi-like appearance of cartilage particles [43].

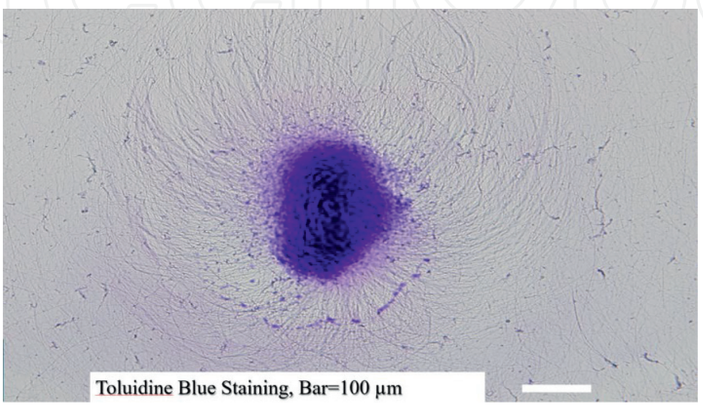


Figure 10.
The cartilage-derived microcarriers stained positively with toluidine blue, and it was strongly positive in the central region near the cartilage particles. The surrounding filament structure is obvious, and it is closely connected with the cartilage particles [43].

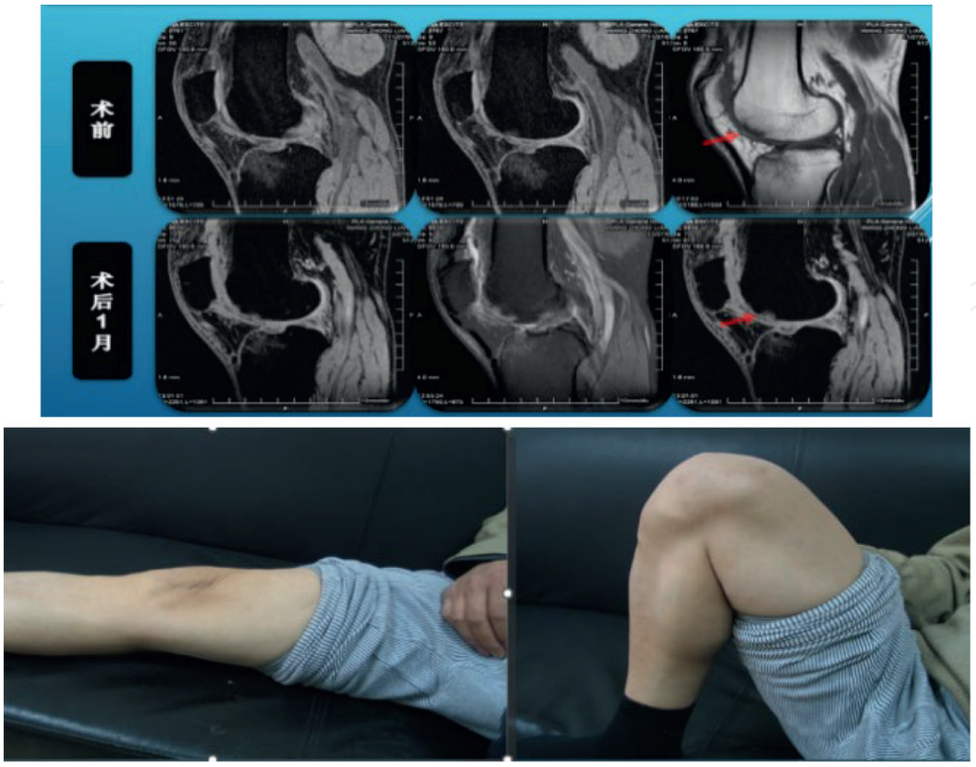


Figure 11.
Source: [45].

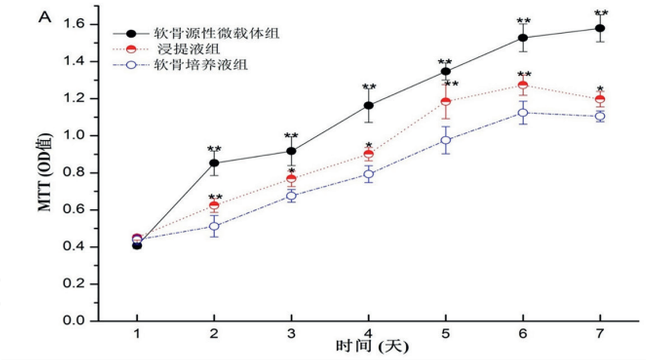
which can provide chondrocytes with a microenvironment that is most in line with the original growth environment of cartilage.

Overall, we can integrate one or more components that are beneficial for the repair of cartilage damage in the microcarrier, provide a microenvironment that is conducive to the growth, proliferation, and differentiation into cartilage of seed cells, and increase the expression of cartilage-related proteins (**Figures 11** and **12**).

6. Suitable supporting matrix

Appropriate supportive matrix is another extremely important reason why cartilage TE can promote cartilage regeneration. We generally divide microcarriers into two categories according to their sources, synthetic microcarriers and natural source microcarriers. The source of synthetic microcarriers is convenient, but their biocompatibility is poor, almost all lack cell-specific recognition sites, and some have some cytotoxicity. Therefore, researchers are now more optimistic about using natural polymers as materials to prepare microcarriers with good biocompatibility, such as gelatin, alginate, chitosan [41].

One study [37] claimed that chitosan microcarriers combined with crocodile dialdehyde bacterial cellulose and DL-allo-hydroxylysine could promote cell proliferation, growth, and migration. Hydroxylysine is a high-quality amino acid with low immunogenicity and good compatibility. Type II collagen contains a large amount of hydroxylysine, and *in vitro* cell culture, hydroxylysine can also promote cell differentiation into cartilage. In addition, oxidized bacterial cellulose can also be used as a raw material for microcarriers, which not only have structural characteristics similar to natural ECM, but also have good physical properties.



Cartilage ECM derived microcarrier can promote the proliferation of chondrocyte; so dose its leaching liquid.

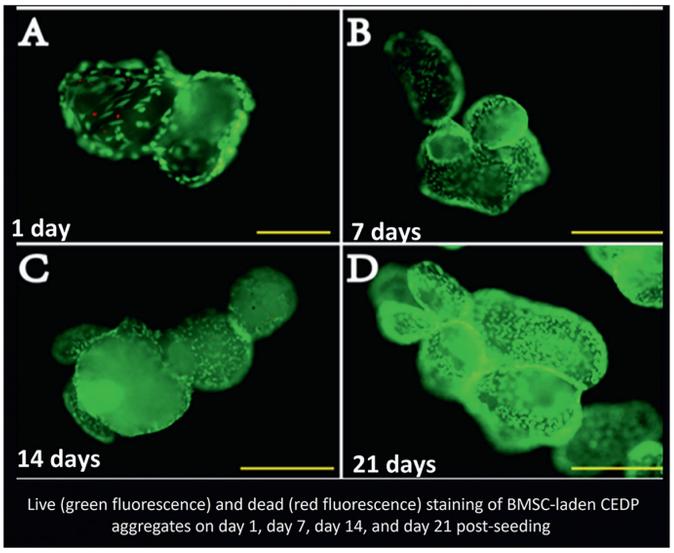


Figure 12.
Source: [42].

In addition, some researchers use both natural and synthetic materials to manufacture the microcarriers. The researchers produced chondrocyte-complexed chitosan polyelectrolyte complex (PEC) microcarriers that significantly outperformed chitosan microcarriers in the ability to generate cartilage matrices. Biomaterials can only be used in the field of cartilage repair if they can promote cell proliferation and differentiation, and have sufficient physical properties. Many scholars have conducted continuous research to make this ideal biological material. Studies by some scholars have shown that porous PLGA microcarriers can promote the formation of cartilage by MSCs. E Filova's team prepared a scaffold of polyε-caprolactone (PCL) porous scaffolds containing chitosan microparticles, which not only possessed good biocompatibility, but also exhibited good mechanical properties. The microcarriers constructed by PCL provide the advantages of sufficiently strong mechanical strength and sufficiently large porosity in cartilage regeneration, and composite chitosan can better promote cartilage repair. And the higher the concentration of chitosan, the better the effect of cartilage repair.

In addition to the above three factors, microcarriers also have many advantages in other aspects (**Figure 13**).

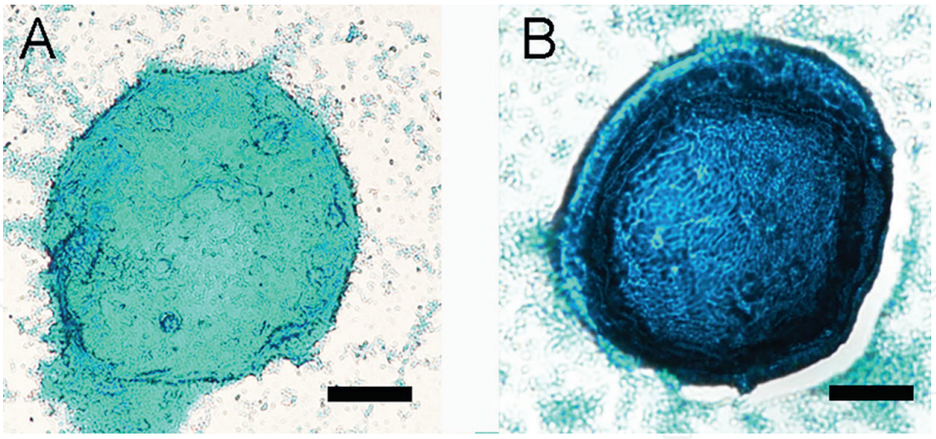


Figure 13.
A is Alcian blue staining of alginate-adipose derived stem cell microspheres after 21 days of chondrogenic induction, blue acid glycosaminoglycan components are visible (scale bar = 100 μm); B is Alcian blue staining of alginate—5 g/L gelatin-adipose derived stem cell microspheres after 21 days of chondrogenic induction, darker acid glycosaminoglycan components were seen (scale bar = 100 μm) [46].

6.1 Microcarriers assist in the formation of microtissues

A study [47] showed that the biocompatibility of the scaffolds was better when the scaffolds were first cultured in a perfusion culture system with exogenous stimulation for a certain period of time and then implanted.

During the construction of tissue-engineered cartilage, the ECM secreted by the cells carried by the microcarriers binds each cartilage microtissue or organoid together, thereby promoting the formation of tissue-engineered cartilage. Seed cell-bound microcarriers were continuously and dynamically cultured in custom-built bioreactors [37]. When the microcarriers begin to secrete ECM, cartilage microtissues can be obtained. In addition, microtissues have other benefits, because functional microtissues can be implanted into the site of cartilage defects, which avoids damage to cell viability and cell numbers caused by the process of digesting cells before the cells are transferred from traditional 2D cultures.

6.2 Microcarriers are injectable

Most of today's tissue engineering treatments for articular cartilage defects require opening the joint cavity to implant bioscaffolds, a process that damages the tissue surrounding the joint. Because of its small diameter, microcarriers can be implanted into the cartilage defect site by injection, thereby reducing the damage to the periarticular tissue caused by the surgical incision [48].

However, in terms of the potential of injectable microcarriers to treat cartilage defects, how to retain or adhere to cartilage defects for a long time after implantation of microcarriers is one of the major issues to be solved. Some studies [37] used bioprotein glue to fix the implanted microtissue in the defect. Although there is nothing wrong with the use of biological protein glue, the microtissue needs to be accurately placed in the cartilage defect, which is difficult to achieve in clinical surgery. Several researchers have developed PLGA microtissues coated with magnetic nanoparticles to enable precise localization of cartilage defects by magnetism. The researchers also prepared alginate-based microtissues that

dispensed MSCs and iron oxide nanoparticles (IONPs) separately, preventing potential damage to cells by IONPs. Another advantage of such microspheres is that they can be loaded with a large number of IONPs, which make them easier to move after magnetization. These magnetic microcarriers play an important role in cartilage repair.

6.3 Incorporating bioscaffolds

Cell-loaded microcarriers can also be used in combination with bioscaffolds for cartilage repair. The combination of PLGA microtissue and collagen/silk fibroin composite scaffold can not only promote cartilage repair, but also promote the fusion of newly formed cartilage with surrounding normal cartilage. It has also been reported that cold atmospheric plasma (CAP)-modified electrospinning scaffolds combined with microtissues can improve the proliferation and differentiation of seeded cells. Another study added hyaluronic acid to the collagen/silk fibroin scaffold, giving it new advantages such as anti-inflammatory and analgesic. At the same time, the researchers added velvet antler polypeptides (PAPs), which can promote cartilage healing, to PLGA microcarriers without seed cells, and found that the scaffolds also promoted the repair of damaged articular cartilage.

6.4 Drug delivery with microcarriers

Microtissues can also be used for drug delivery. Some researchers have repaired pig cartilage by using microtissues to deliver BMP-2 and TGF-3 at the site of injury and release them continuously. One study [49] demonstrated that microtissues containing chondroitin sulfate, a drug that favors cartilage repair, were superior to microfracture techniques for cartilage regeneration. The use of microcarriers as an injectable drug delivery system is not only feasible, but also simple and easy to implement, and effective in the treatment of cartilage damage [50, 51] .The authors [50] infiltrated PLGA microtissues in fluvastatin, which had a significant effect on reducing cartilage degeneration in rabbits. They [51] added tumor necrosis factor-α-stimulated gene 6 in PLGA microtissues and achieved obvious effects in repairing cartilage defects in rats.

In conclusion, microcarriers have multiple and distinct advantages in cartilage repair.

7. Prospects Application prospect of microcarriers in the treatment of cartilage injury

The ability of microcarriers to provide a suitable microenvironment is very important for the repair of cartilage defects. In addition, some studies [37] have shown that microcarriers whose structures are more similar to the cartilage tissue's native ECM perform better in repairing articular cartilage damage. In addition, other studies [48] have shown that there is a higher rate of cell attachment if the cartilage tissue microcarriers contain ECM. Therefore, we reasoned that if the native ECM could be directly incorporated or generated in microcarriers with appropriate physical characteristics, the effect of cartilage repair would be improved.

It is also an unsolved problem at this stage to find raw materials for the production of microcarriers that can perfectly replace the natural cartilage ECM. At present,

there is also a lack of research on whether microcarriers prepared by composite materials can effectively promote the growth, proliferation, and differentiation of seed cells.

Physical properties such as porosity and volume have become key factors affecting the efficacy of cartilage repair. The future development direction of microcarriers should be how to determine the appropriate porosity and volume according to the specific conditions of patients, and establish an efficient way to calculate these physical properties, which will play an important role in future clinical applications.

The source of cells is also an important condition that affects the application effect of microcarriers. The source of chondrocytes is often the patient's own, and obtaining chondrocytes will damage the cartilage. In contrast, ADSCs may be a better choice. Another point to study is how much the patient's age, disease, etc., affect the quality of the obtained seed cells.

Injectable microcarriers allow the treatment of cartilage injuries to be performed minimally invasively, reducing damage to surrounding tissue from the incision. The research and development of magnetic microcarriers has greatly solved the problem that it is difficult to accurately locate cartilage defects by injection methods [37]. If we continue to conduct more in-depth research in this area, or find other methods that can accurately locate under injection treatment conditions, the treatment of cartilage injury with microcarriers is one step closer to clinical application.

Although many *in vivo* and *in vitro* experiments have demonstrated that microcarriers can repair cartilage well, they have not yet reached the clinical trial stage. Orthopedic surgeons who want to pay attention to OA treatment can continue to pay attention to the research progress of microcarriers and confirm their ability to repair human cartilage through clinical studies.

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